INDUCTION OF HEMOXYGENASE 1 PREVENTS ACUTE HEPATIC CHOLESTASIS PRODUCED BY OXIDATIVE STRESS IN THE RAT

PAMELA MARTIN; PAULA CECCHATO; SANDRA ARRIGA; ENRIQUE SANCHEZ POZZI; MARCELO ROMA; ECCELIA BASILIO
Instituto de Fisiología Experimental (IFISE - CONICET); Área Bioquímica Clínica, Facultad de Ciencias Bioquímicas y Farmacéuticas, UNR, Rosario, Argentina.
martins@ifise-conicet.gov.ar; basiglio@ifise-conicet.gov.ar

INTRODUCTION: Cholestasis is defined as the reduction of bile flow and the consequent accumulation of toxic compounds in liver and blood. Under oxidative stress (OS) conditions, reactive oxygen species (ROS) are generated due to mitochondrial damage. Bilirubin (BR) is an endogenous bilary pigment derived from heme metabolism by enzymes hemoxygenase 1 (HO1) and biliverdin reductase. In a previous study, we demonstrated that un conjugated BR exerts an important protective effect on biliary secretory failure induced by OS, even at physiological concentrations.

MATERIALS AND METHODS: Wistar rats (♀) were divided into 4 groups: Control: treated with DMSO (vehicle); tert-butyl hydroperoxide (tBBOOH) treated with 240 μmol/kg.p.c. Bsep (proteasolic agent), and p.Hemin: treated with 20 mg/kg.p.c. Hemin (HO1 inducer), i.p., and Hemin + tBBOOH: treated with 20 mg/kg.p.c. heman i.p. + 440 μmol/kg.p.c. IBOOH i.p. At 4 h post IBOOH treatment, bile flow was monitored and bile samples were collected every 10 minutes for 2 h. After euthanasia, liver tissue samples were collected and stored (−70°C).

ABLE: To study the effect of HO1 induction and the consequent increase in endogenous levels of BR on the hepatocellular redox status and the function of two key hepatocanalaric transporters, Bsep and Mrp2.

HYPOTHESIS: Hepatic diseases bearing an oxidative background would have a more severe outcome in terms of hepatobiliary function in the absence of BR, and the modulation of endogenous BR levels would have a beneficial effect on the course of oxidative cholestatic pathologies.

RESULTS:

Oxidative damage to lipids and proteins was assessed in liver tissue by the reaction of the Thioarbituric Acid Reactive Substances (TBARS) and the determination of protein carbonyls by the Levine method, respectively. Antioxidant defenses were evaluated in liver tissue through CAT activity, measured by spectrophotometrically monitoring the disappearance of H2O2 at 240 nm (Bears and Sizer method), and SOD activity, measured using a commercial kit (Random, Crumlin, UK). In bile samples, GSH and GSSG were measured by the Griffith method (modified by Tietze), as a sensible marker of OS.

Biliary secretory function was evaluated through the determination of biliary excretion of bile salts (Bsep substrates), by the Talalay method (modified by Berthelot) and biliary excretion of GSH (Mrp2 substrate), as described above. Localization of hepatocanalaric transporters was studied in liver tissue samples through fluorescence confocal microscopy.

CONCLUSION: Induction of HO1 and consequent elevation of BR levels protect the liver from oxidative injury, thus contributing to limit the progression of cholestatic liver diseases that concurs with OS.